

RCM

Letter to the Editor

Dear Editor,

The successful inclusion of succinylacetone as a marker of tyrosinemia type I in Tuscany newborn screening program

Tyrosinemia type I (MIM 276700) is an autosomal recessive disorder due to the deficiency of fumarylacetoacetic hydrolase (EC 3.7.1.2), the last enzyme in the tyrosine metabolism. This condition, if not treated, is characterized by severe liver failure and renal and neurological involvement.¹ The availability of an effective treatment has increased the need to improve early detection and has made this disease an eligible candidate for

newborn screening by flow injection analysis tandem mass spectrometry (FIA-MS/MS).² Up to now, tyrosine has been the common marker for tyrosinemia type I, causing many false positive and false negative results.^{3,4} Indeed a great number of affected infants have normal tyrosine levels at the time of the routine heel-stick for newborn screening. To better detect tyrosinemia type I through newborn screening, succinylacetone (SA), the primary diagnostic marker for tyrosinemia type I, has been recently introduced into the panel of the metabolites tested.^{5,6}

Since January 2007, 87 700 newborns from Tuscany have been screened using FIA-MS/MS by a modified method including SA, as reported by la Marca.⁵ During this period, we diagnosed an infant with tyrosinemia type I through newborn screening. The patient, a female, is the second child of first-cousin Turkish parents. Their first son, born after three consecutive miscarriages, is healthy. She was born at term (Apgar score 9^I to 10^V) with a weight of 3150 g after an uneventful pregnancy and delivery. Neonatal screening revealed a SA value of 7.6 $\mu\text{mol/L}$ (n.v. <2) and normal tyrosine values (126 $\mu\text{mol/L}$; n.v. <250) (Fig. 1). She was admitted to hospital and diagnosis of tyrosinemia type I was

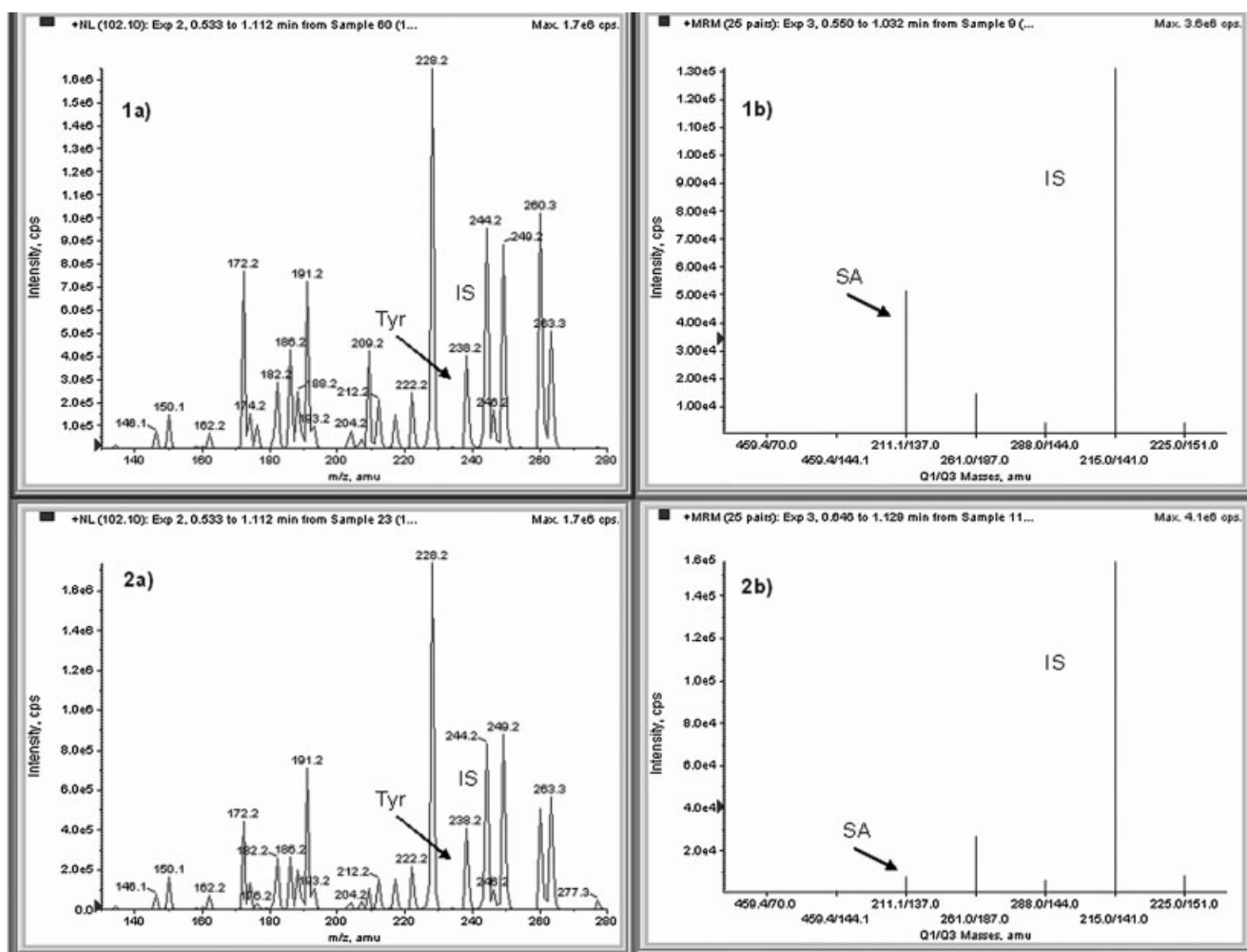


Figure 1. Panel 1: the patient affected by tyrosinemia type I identified through newborn screening. Tyrosine levels are within the normal ranges (1a) whereas high levels of SA are detected (1b). Panel 2: control with tyrosine (2a) and SA (2b) levels within the normal ranges.

confirmed on a second dried blood spot (DBS) and urine. On the fifth day of life the child was in good clinical condition and no clinical signs of hepatopathy were noted. Treatment with 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) (1 mg/kg/day) and a low protein intake were started.

As reported in our previous work,⁵ SA was coextracted with acylcarnitines and amino acids by using a mixture of hydrazine hydrate (1 mmol/L) in water and methanol. Modifications of the routinely newborn screening procedure previously used and described by us⁷ were limited to extraction time extended to 25 min instead of 20 min and to extraction temperature increased up to 37°C instead of room temperature. The derivatization phase was not modified. Several hydrazine concentrations (up to 24 mmol/L) were tested. As the concentration of hydrazine becomes higher, ionization efficiency decreases and hydrolysis and interfering components increase. The choice to use hydrazine hydrate instead of the commercially available hemisulfate, sulfate or dihydrochloride salts was made to avoid production even only of a small amount of sulfuric acid and hydrochloric acid. Moreover, 1 mmol/L concentration of hydrazine was used because it could extract succinylacetone without any significant interferences with the analytical

performance and with the quantitative assay. The final pH of the solution is 4.4 and is not strong enough to cause acid hydrolysis of ester groups. Hydrazine hydrate solution does not modify acylcarnitine and amino acid concentrations, as demonstrated by la Marca.⁵ The detection of butylated SA and ¹³C₄-SA (internal standard) hydrazones was performed by two MRM transitions *m/z* 211→137 and *m/z* 215→141, respectively. One SA-spiked DBS (5 μmol/L) is run every day to ensure that the SA extraction procedure has come to completion. In this patient, the diagnosis of tyrosinemia type I was confirmed by a modified high-performance liquid chromatography (HPLC) method⁸ on DBS and urine specimens. The modification concerned the use of hydrazine hydrate solution (1 mmol/L) instead of dansylhydrazine solution (2.5 mmol/L) during the first derivatization step. The resulting SA level was 10.0 μmol/L on the second DBS (v.n. <2) (Fig. 2) and 92.3 μmol/mmol of creatinine (v.n. 0.006–0.14) in urine.

This case demonstrated that the inclusion of SA in newborn screening programs improves the effectiveness to identify infants affected by tyrosinemia type I. A variety of sample preparation methods are reported to determinate SA on DBS.⁹ As far as we know, the elevation of blood

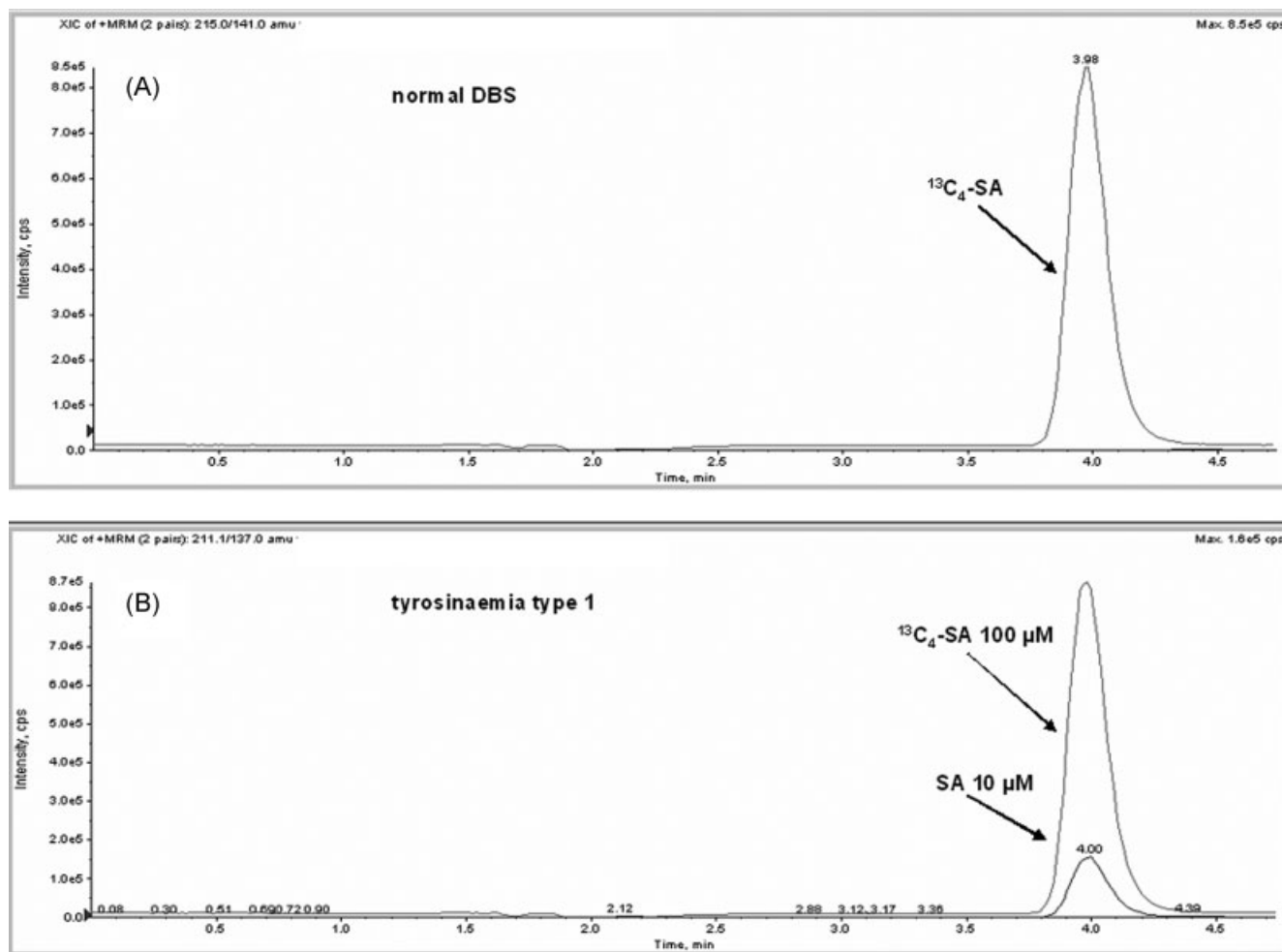


Figure 2. Confirmation test by using a HPLC method. Extracted ion chromatogram of a normal DBS whereas only internal standard (IS) is detected (a); extracted ion chromatogram of the affected newborn DBS at recall (b). A clear signal corresponding to SA is well depicted under the IS peak.

succinylacetone detected by newborn screening has been described only once prior to this report in two newborns, and was identified on residual spots, after extraction of acylcarnitines and amino acids, by separate runs.¹⁰ Our method allows SA to be extracted simultaneously with acylcarnitines and amino acids. The application of this method, which has proven to be accurate and reliable, offers many advantages including time saving and no additional costs. No second tier test is needed. We believe that the SA determination should be performed in all newborn screening programs.

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